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A STUDY OF METHODS SUITABLE FOR THE ANALYSIS AND PRESERVATION OF PHOSPHORUS FORMS IN AN ESTUARINE ENVIROMENT

David Jenkins

California University

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A STUDY OF METHODS SUITABLE FOR THE ANALYSIS AND PRESERVATION OF PHOSPHORUS FORMS IN AN ESTUARINE ENVIRONMENT

BY
David Jenkins

November 1965

Sanitary Engineering Research Laboratory
College of Engineering
and
School of Public Health
University of California
Berkeley

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A STUDY OF METHODS SUITABLE FOR THE ANALYSIS AND PRESERVATION OF PHOSPHORUS FORMS IN AN ESUTARINE ENVIRONMENT

I. INTRODUCTION

The study described in this report was conducted under a purchase order from Region IX of the United States Public Health Service, Water Supply and Pollution Control Division, San Francisco, to the Regents of the University of California.

II. OBJECTIVES

The objectives of the study were:

1. To review the literature on techniques of phosphorus analysis

2. To determine the suitability of suggested analytical techniques for the estimation of phosphorus forms in an estuarine environment

3. To determine the precision and accuracy of the selected techniques of analysis

4. To determine the most suitable method of preservation of samples to be analyzed for various forms of phosphorus.

III. METHOD OF PROCEDURE

1. Sample Collection

Sampling points were selected in the San Francisco Bay System to give water samples typical of:

a. Fresh water inflow

b. Brackish water

c. Offshore saline water

A summary of the chlorosity and suspended matter content of the samples, analyzed immediately after sample collection, is given in Table I. For samples used in the method development work a large quantity of each sample was Millipore filtered shortly after the samples arrived in the laboratory. Filtered and unfiltered portions were then stored in the refrigerator at 4°C. A brackish water sample was used for investigation of preservation methods and was taken from a single sampling point. The sample used in the preservation study was collected early in the morning and brought to the laboratory immediately so that initial analyses could be performed on the same day as sampling. A large volume of sample was collected and after mixing thoroughly was split into smaller portions, placed in glass bottles, and treated with the desired preservative.

TABLE I

SUSPENDED MATTER AND CHLORIDE CONTENT OF SAMPLES
USED FOR METHOD DEVELOPMENT

Sample Location	Suspended Solids mg/ℓ	Chloride mg Cl^- /ℓ
Pittsburg	74	810
Crockett	38	7600
Fort Baker	10	19500

2. General Laboratory Procedure

The forms of phosphorus studied were as follows:

a. Soluble orthophosphate

b. Soluble condensed phosphate

c. Total soluble phosphorus

d. Total phosphorus

e. Available phosphorus (a limited study on the availability of phosphorus associated with the suspended material.)

The methods of analysis that appeared to be most suitable were selected by a review of the literature. These methods were then tested in the laboratory for accuracy, reproducibility, recovery, and presence of interferences using the three Bay water samples from areas of differing salinity. Following the development of a satisfactory technique of analysis for each of the aforementioned forms of phosphorus, a series of preservation experiments was conducted to determine the most suitable method of sample preservation.

IV. RESULTS OF STUDY

1. General Schemes of Phosphorus Analysis

In general, the analysis of all forms of phosphorus can be divided into two separate parts. The first part of the analysis usually consists of a preliminary treatment in which the phosphorus form of interest is separated and converted into the orthophosphate form. In the second part of the analysis the orthophosphate is determined by a colorimetric technique.

For the purposes of this study, phosphorus was classified into six separate categories. A chemical classification into orthophosphate, polyphosphate and organic phosphorus was made and the corresponding physical separation of these three chemical forms into soluble and insoluble components was made.

The separation of soluble and insoluble forms is made by some means of filtration or centrifugation. In the analysis of the various chemical forms of phosphorus, the technique of sample treatment used to convert the phosphorus form of interest to orthophosphate varies with the phosphorus form. Since both organic and inorganic condensed phosphates are sensitive to decomposition by some of the reagents used in the analysis of orthophosphate, care has to be taken that no decomposition of these condensed or combined forms takes place during this analysis. Polyphosphate and other condensed phosphates (e.g. meta - and pyrophosphates) can be converted to

orthophosphate by boiling in an acidic solution. In order to decompose the C-O-P bonds encountered in organic matter, it is necessary to employ a more drastic decomposition technique to form orthophosphate. Boiling with perchloric/nitric acid mixtures, percholoric acid alone, ashing at 800°C in the presence of magnesium chloride, and digestion with persulfate have been suggested as methods of decomposing organically bound phosphorus.

The colorimetric estimation of orthophosphate almost universally involves the formation of phosphomolybdic acid which is then reduced with one of a variety of reducing agents to molybdenum blue, the concentration of which is then estimated colorimetrically.

2. Initial Separation into Soluble and Insoluble Forms

In this study, the initial separation of phosphorus forms into soluble and insoluble fractions was carried out by using Millipore filters (Type HA, 0.45μ, white grid, 47 mm diameter). Since a whole spectrum of particle sizes are undoubtedly present in San Francisco Bay water, the apparent quality of soluble or insoluble phosphorus forms depends largely on the pore size of the filter used. Thus, Rigler [1] found that the percentage of soluble organic phosphorus in three lake water samples decreased progressively as the pore size of the filters was reduced from 5μ to 0.1μ (Table II).

TABLE II

EFFECT OF SEPARATION METHOD ON AVERAGE PER CENT OF TOTAL PHOSPHORUS APPEARING AS SOLUBLE ORGANIC PHOSPHORUS IN THREE LAKE WATERS (after Rigler [1])

Millipore filter modal pore size μ	Average soluble organic phosphorus - per cent of total phosphorus
5.00	42
1.20	37
0.45	33
0.22	21
0.1	18

It is generally agreed that the HA Millipore filter provides a reasonable compromise between slow filtration rates and poor particle retention. It should be borne in mind, however, that 0.45μ does not represent the effective pore size which exists shortly after the commencement of filtration when a sediment pack begins to form on the filter. For this reason, some colloidal material with a diameter of less than 0.2μ may be retained on the filter. Whether or not large diameter phosphorus containing molecules (diameter ~5mμ) are retained on the filter has not been established.

It is therefore important to state that when the terms soluble and insoluble are used in this report to define a certain type of phosphorus, the strict connotation should be "Millipore (0.45μ) filterable" for insoluble and "passing a Millipore filter (0.45μ)" for soluble.

Another consideration which must be taken into account when using Millipore filters for the separation of soluble and insoluble forms is the presence of releasable phosphorus in the Millipore filter. Rigler [1] found that Millipore filters of the type used in this work contained about 1μg of phosphorus per filter which would be released when distilled water was passed through the filter. In an experiment carried out during the present study, three 100 ml portions of distilled water were passed consecutively through an unwashed Millipore filter and then analyzed separately. The first 100 ml portion contained 0.97μg P, the second portion contained 0.14μg P, and the third portion was free of phosphorus. When unwashed Millipore filters were completely digested in a nitric acid/perchloric acid mixture, they were found to contain 1.32$\pm$ 0.06μg P per filter. On the basis of these findings it is recommended that Millipore filters be soaked in distilled water for 24 hours before use in any separative technique preceeding a phosphorus analysis. It is possible to soak at least 40 filters in each liter of distilled water. If the distilled water used for soaking the filters is changed after the first hour, it was shown that a further three hours soaking in fresh distilled water was adequate. Therefore if time is of the essence, the 24-hr soaking period in one batch of distilled water can be replaced by a 4-hr soaking period in which the distilled water is changed after the first hour's soaking.

3. Condensed Phosphates

For the purposes of this study the term condensed phosphate means those organic and inorganic phosphates capable of being hydrolyzed to orthophosphate under mild acid conditions. In particular, this would include pyrophosphate ($P_2O_7^{-4}$), tripolyphosphate ($P_3O_{10}^{-5}$), and the various metaphosphates $[XPO_3]n$.

The analytical scheme for the separation of various phosphorus forms in San Francisco Bay would be greatly simplified if one could assume that the amount of condensed phosphates present was negligible. The only significant source of condensed phosphates in the Bay water would be from waste discharges containing polyphosphates derived from detergent products. Since the rate of hydrolysis of polyphosphates to orthophosphates is known to be rapid under the conditions in natural waters one might surmize that the concentration of polyphosphate should be low in the waters of the Bay. It was therefore decided that the first analytical effort should be directed toward the determination of condensed phosphate levels in the samples.

Samples from the three areas of the Bay were analyzed for condensed phosphate by the American Association of Soap and Glycerine Producers' (AASGP) method [2] . This procedure uses a mild acid hydrolysis to break P-O-P bonds while P-O-C and P-C bonds remain intact. After hydrolysis, a colorimetric analysis of orthophosphate is performed using the isoamyl alcohol/benzene extraction technique discussed in the following section on soluble orthophosphate. The results of this work are given in Table III.

TABLE III

ORTHO AND HYDROLYZABLE CONDENSED PHOSPHATE ANALYSES ON SAMPLES TYPICAL OF FRESH, BRACKISH, AND SALINE WATER FROM SAN FRANCISCO BAY

Sample Location	Suspended Solids mg/ℓ	Chloride mg Cl^-/ℓ	Ortho-phosphate µg P/ℓ	Ortho + Hydro-lyzable Phosphate µg P/ℓ	Hydro-lyzable Phosphate µg P/ℓ
Antioch	155	130	44.7	48.6	+3.9
Crockett	408	5,400	62.6	58.4	-4.2
Fort Mason	34	16,700	64.3	63.4	-0.9

It can be seen that the amount of orthophosphate found before and after hydrolysis is almost identical. In the one sample which shows an increase in orthophosphate after hydrolysis, this increase is less than 4 µg P/ℓ. From these results it was concluded that the amount of condensed phosphates present in the Bay waters was insignificant and that the detection and estimation of this form of phosphate could be dropped from the analytical scheme.

4. Soluble Orthophosphate

a. General Outline of Method

The analysis of orthophosphate almost universally relies on the formation of phosphomolybdic acid in acidic solution by the reaction of the orthophosphate with ammonium molybdate. The ammonium salt of the phosphomolybdic acid complex formed by this reaction has a yellow color which can be used for the quantitative determination of orthophosphate but usually lacks sufficient intensity to be of practical value. The phosphomolybdic acid complex is therefore reduced by one of a variety of reducing agents, the most common being stannous chloride, various isomers of aminonaphtholsulfonic acid, ascorbic acid and benzidine. The reduction of the phosphomolybdic acid to molybdenum blue can be carried out in aqueous solution or in an organic extract of the solution which contains the phosphomolybdic acid. Various solvents have been suggested for the extraction of phosphomolybdic acid from the aqueous phase, the most common being isobutyl alcohol.

The method selected for use on San Francisco Bay water is a modification of a technique suggested by the AASGP [2]. In this method the filtered water sample is reacted with ammonium molybdate in acid solution and the resulting phosphomolybdic acid complex is extracted into an isoamyl alcohol/benzene mixture. The extracted phosphomolybdic acid is reduced with stannous-chloride to molybdenum blue which is then measured colorimetrically.

b. Filtration of Samples

Early in the investigation it was found that orthophosphate analyses carried out on unfiltered samples containing suspended material gave anomalous and unreproducible results. Further study of this behavior revealed that the amount of orthophosphate which could be recovered from a sample containing suspended solids depended on how long and how vigorously the samples was shaken during the analytical procedure. It appeared that during the analysis, orthophosphate which was either adsorbed on or associated with the sediment, was being released into solution. In addition to the extraction of orthophosphate from suspended sediment, variation of salinity was found to affect color development in the sample. Considering these two interferences and the fact that large turbidity corrections

were necessary when samples high in suspended solids were analyzed, it was decided that only an estimation of soluble orthophosphate should be made.

It has been established by previous work in this laboratory that orthophosphate does not adsorb on the Millipore filter or on the sediment layer formed on the filter during filtration.

c. Method Development

While the basic AASGP method [2] was adopted for the analysis of soluble orthophosphate, investigations were carried out to determine the effects of varying chloride concentration as well as the effect of other interferences likely to be encountered in the analysis of San Francisco Bay water.

The solvent used for the extraction of the phosphomolybdate complex from the aqueous phase was a 1:1 mixture of benzene and isoamyl alcohol, rather than the benzene/isobutyl alcohol solvent recommended by AASGP [2]. The solubility of isoamyl alcohol in water is about one-fifth that of isobutyl alcohol. Therefore, when large volumes of aqueous sample are being extracted using the reagent suggested by AASGP [2], it is necessary to add extra isobutyl alcohol to the mixture in order to replace that which dissolves in the aqueous phase. The use of the less soluble isoamyl alcohol obviates the need for this step in the procedure. In the analysis of 20 µg orthophosphate -P in the presence of 20 g Cl^-/ℓ, it was noticed that the absorbance produced was depressed slightly over that obtained when 20 µg orthophosphate -P was analyzed in distilled water. When using benzene/isobutyl alcohol as the organic phase, the depression of absorbance was slightly less than 5 per cent. A benzene/isoamyl alcohol extract produced a depression of about 2.8 per cent. While the depression of absorbance due to 20 g Cl^-/ℓ is small, and the significance of the difference between the depressions has not been established, it is thought to be wiser to pick the solvent mixture that produces the smallest chloride effect.

The depression of absorbance caused by 20 g Cl^-/ℓ was demonstrated to vary with the amount of orthophosphate present in the sample subjected to extraction. Thus, 20 g Cl^-/ℓ produced 4.5 per cent, 2.8 per cent, and 0.5 per cent respectively for sample contents of 5 µg, 20 µg, and 50 µg PO_4 - P. These per cent depressions represent approximately the same absolute amount of PO_4-P.

From experience it has been found that 200 ml samples of sea water contain about 20 μg PO_4-P/sample. Thus, depressions of 2-3 per cent may be expected in water of a salinity equivalent to about sea water concentration. This deviation can be tolerated.

Notwithstanding the small depression in PO_4-P produced by 20 g Cl^-/ℓ, it was decided to study the effect of varying the proportions of isoamyl alcohol and benzene in the solvent mixture on the interference of chloride.

First, solvent mixtures consisting of 20 ml/30 ml, 30 ml/20 ml, 15 ml/35 ml and 25 ml/30 ml isoamyl alcohol/ benzene were checked for chloride interference by analyzing 20 μg PO_4-P in a distilled water medium containing 30 g Cl^-/ℓ. The solvent mixture containing the 25 ml/30 ml mixture of isoamyl alcohol/benzene showed the least depression of absorbance--1 per cent at 2 min reading time and 1.5 per cent at 10 min reading time.

In a second experiment on the same distilled water medium using solvent mixtures of 20 ml/25 ml, 25 ml/25 ml (the recommended ratio) and 25 ml/30 ml, the latter solvent mixture again showed superior results giving a 1 per cent depression for a 2 min reading time and 2.3 and 4.2 per cent depressions for 10 min and 30 min reading times, respectively. Using the 25 ml/30 ml solvent mixtures for the analysis of 20 μg PO_4-P in a distilled water medium containing 20 g Cl^-/ℓ depressions of 1.2 per cent, 1.2 per cent, and 2.4 per cent were found for 2 min, 10 min, and 30 min reading times respectively.

It would therefore seem that, in the matter of chloride depression, the 25 ml/30 ml solvent mixture is superior to the presently recommended 25 ml/25 ml mixture.

Since this study was made at the end of the Investigation, the former solvent mixture is not recommended in this report because shortage of time prevented a thorough check of other interferences and of replication using this solvent system.

A further advantage of isoamyl alcohol over isobutyl alcohol is the 10-15 per cent increase in sensitivity obtained with the former. Although the price of isoamyl alcohol is higher than that of isobutyl alcohol, a smaller volume per test is required thus the increased solvent cost amounts to only about 3¢ per analysis, from about 6-7¢ for isobutyl alcohol to 9-10¢ for isoamyl alcohol.

The use of pear-shaped separatory funnels is recommended for the colorimetric determination of orthophosphate. This type of funnel provides a greater depth of organic phase and therefore increases the ease with which an aliquot can be removed.

Teflon plugs are recommended since they are resistant to the solvents used in the analysis and can be used without stopcock lubricants which may dissolve in the organic phase and interfere in the analysis.

The temperature of extraction was found to exert little or no effect on the results in a range of 15-30°C. At 5°C and 40°C an increase and decrease respectively of about 2 per cent in the absorbance obtained at room temperature (25°C) occurred.

d. Interferences

The method was investigated rigorously for possible interferences that might influence the results when applied to estuarine waters.

As previously mentioned, chloride ion exerted a small (and we feel negligible) depressive effect on the absorbance of a given amount of orthophosphate.

The hydrolysis of condensed phosphates during the orthophosphate analysis is virtually eliminated by minimizing acid contact time with the sample. Previous work in this laboratory has shown that negative results were obtained with this technique when analyses were carried out on concentrated solutions of sodium tripolyphosphate, while in the reactive phosphate technique [3] (used previously in this laboratory), considerable hydrolysis of the polyphosphate was found to take place.

Of the elements which may form heteropoly acids under similar conditions to those used in the proposed method (As^{+5}, Si^{+4}, Ge^{+4}, Mo^{+6}, and B^{+3}) only arsenic and silicon are likely to be present in estuarine waters in significant concentrations.

Under the pH conditions recommended, it was found that 50 mg SiO_2/ℓ (which is more than twice the maximum concentration found in San Francisco Bay waters) gave a negligible absorbance when carried through the test procedure.

Contrary to expectation, since it is listed in most phosphate methods as being an interfering ion, arsenate was found to have no effect on the recommended procedure. As much as 500 µg AsO_4^{-3}-As/ℓ in either distilled water or sea water (with and without the presence of 20 µg PO_4-P) had no effect on the final absorbance obtained. When a phosphate method which did not use an extraction step, was tested (reactive phosphate), it was found that 50 µg AsO_4^{-3}-As gave an apparent phosphate concentration of 23.4 µg P. No clear explanation can be made for the absence of arsenate interference, but it may be surmized

that the concentration of ammonium molybdate and the pH are unfavorable for arsenomolybdic acid formation or that the arsenomolybdic acid may be insoluble in the organic phase.

Copper and iron, which may be released into solution by the digestion of sediments in the analysis of total insoluble phosphorus, were found not to interfere in concentrations (200 µg Cu^{+2} and 3 mg Fe^{+3}/ sample) several times greater than those found in typical Bay sediments previously analyzed in this laboratory.

Other common interferences in the analysis of phosphate given in the literature are S^{-2}, tannins, lignin, and Cr^{+6} but none of these materials is likely to be present in Bay water in concentrations that would cause interferences.

Since mercuric chloride (40 mg Hg^{+2}/ℓ) and chloroform (5 ml $CHCl_3$/ℓ) had been chosen as preservatives which may be suitable for treating samples to be analyzed for phosphorus forms, they were tested for possible interference in the analytical determination of orthophosphate.

Using 20 µg PO_4-P per sample it was found that at the aforementioned levels, mercuric chloride had no effect on the recovery of orthophosphate however a small decrease in recovery occurred when chloroform was added to the solution. This is a logical result because chloroform contributed additional volume to the organic layer.

e. Precision and Accuracy

Replicate analyses of soluble orthophosphate in waters from three points in the Bay of widely varying salinity were performed by the technique recommended in Appendix IB and the results are presented in Table IV. The precision is excellent; in all cases the coefficient of variation is less than 1.5 per cent.

With a 1 inch light path, and using the reagents and spectrophotometer specified in Appendix IB, the method usually gave a reagent blank of 0.000 absorbance units while 20 µg P produced an absorbance of about 0.290 units. Beer's law was obeyed over a wide range so that 50 µg P/sample can be analyzed without aliquoting of the sample. The limit of detectability was approximately 0.5 µg P. Thus, using a 200 ml sample, 2.5 µg P/ℓ can be determined at the limit of the method.

Notwithstanding the chloride effect noted earlier, it was found to be possible to recover quantitatively 5 µg and 20 µg PO_4-P added to a Bay water sample from Fort Mason which contained approximately 19 g Cl^-/ℓ.

TABLE IV

REPLICATE ANALYSES OF SOLUBLE ORTHOPHOSPHATE
IN SAN FRANCISCO BAY WATER

	Location of Sampling		
	Pittsburg	Crockett	Fort Baker
µg P/ℓ	48.9 47.6 48.3 47.6 48.0 47.3 48.9 48.3 48.0 48.3	76.1 77.6 76.1 75.7 77.0 76.6 75.4 75.4 76.6 76.1	92.6 95.3 92.6 95.9 95.6 94.6 95.3 93.2 92.6 94.3
Mean, µg P/ℓ	48.3	76.4	94.4
Standard Deviation, µg P/ℓ	0.565	0.744	1.30
Coefficient of Variation %	1.17	0.97	1.38

5. Total Soluble Phosphorus

a. General Outline of Method

A determination of total soluble phosphorus will include soluble orthophosphate (soluble condensed phosphates if present) and soluble organic phosphorus. The method of pretreatment will necessarily be some form of oxidation (and hydrolysis) of a Millipore-filtered sample which will release phosphorus as orthophosphate from its combination with organic matter as a C-O-P bonded state and will hydrolyze any condensed phosphate present to orthophosphate.

b. Method Development

A number of oxidants and techniques have been suggested in the literature for this purpose, viz. ignition [4], digestion with 30 per cent H_2O_2 [5], acid treatment (hydrolysis) at 30-40 psi [6], nitric acid/sulfuric acid digestion [2], perchloric acid digestion [7], digestion with a perchloric acid/nitric acid mixture [2], and digestion with potassium persulfate [8].

Each of the above digestion techniques (with the exception of acid hydrolysis at 30-40 psi which requires special equipment not worthy of purchase solely for this purpose) have been studied for their applicability to the analysis of total soluble phosphorus in San Francisco Bay water. With the exception of the persulfate digestion all methods have been found to be unsatisfactory or impractical for the analysis of samples with high dissolved solids contents. Any digestion technique that causes a significant volume reduction (i.e., ignition, perchloric acid digestions, nitric acid/sulfuric acid digestions) results in the formation of precipitates and also in spattering. Thus 200 ml samples (which is the volume required for the determination of low amounts of total soluble phosphorus) of a composition approximating sea water will produce about 7 g residue if evaporated to dryness as in an ignition.

Furthermore, there is some evidence that at the temperatures used in ignition (800°C) the salt melt produced will leach phosphorus from the crucible containers.

In perchloric acid and nitric acid/sulfuric acid digestions, salt precipitates out as the digestion reduces the volume of solution. Danger exists that dry spots may be formed in a heavy crystal mass during perchloric acid digestions and so create a hazard of explosion. In this procedure chloride ion is replaced by perchlorate ion and thus the acidity of the solution is reduced. Since the colorimetric technique for orthophosphate, which follows the digestion, relies on the

presence of a certain amount of acid, it is necessary to adjust the volume of perchloric acid used according to the salinity of the sample. This procedure is a tedious one that does not lend itself to adoption for the routine analysis of large numbers of samples.

The use of hydrogen peroxide (30%) in a boiling solution as suggested by Jackson [5], was found to yield incomplete recoveries of total soluble phosphorus from all Bay water samples tested and from organic media such as Difco tryptone and Difco peptone. Moreover the method failed to yield quantitative recovery of an orthophosphate standard run through the test procedure.

It can be surmized that the low result for orthophosphate standard was due to the presence of unreacted peroxide remaining in the reaction mixture which was treated colorimetrically. If this were the case, peroxide would react with the molybdic acid to form permolybdic acid (Table V).

The peroxide digestion method of Jackson [5] was modified by evaporating a 200 ml sample to dryness on a steam table in the presence of 20 ml 30% H_2O_2, drying the residue further in a 105°C oven, adding 25 ml 8N H_2SO_4 and heating again on a steam table for 30 minutes. The solution was then cooled and analyzed for orthophosphate. By this technique recoveries of total soluble phosphate were good from Bay waters and from an orthophosphate standard but only fair from one of the organic media tested (Table V). While the modified peroxide method did show some promise, its development was not pursued in the light of the extremely satisfactory results obtained by the persulfate digestion technique [8] which, besides its excellence in performance, had a simplicity in execution that made it especially attractive for routine application.

In the persulfate digestion technique the phosphorus present in soluble organic materials is oxidized to orthophosphate while the oxidizing agent, persulfate, reduces to sulfate. It was at first thought that the production of hydrogen peroxide as well as the oxidation of Cl^- to Cl_2 by this oxidizing agent would have serious consequences in the colorimetric orthophate analysis. However, no interferences from these sources could be detected in the colorimetric estimation of orthophosphate as evidenced by the complete recovery of orthophosphate, of soluble organic phosphorus in tryptone (both in the presence and absence of 20 g Cl^-/ℓ) and in samples from three points in San Francisco Bay (Table V). The persulfate oxidation is carried out by autoclaving the sample in the presence of persulfate at 15-20 psi for 1 hr. After cooling, the samples are analyzed for orthophosphate by the procedure given in Appendix IB.

TABLE V
COMPARISON OF SEVERAL TECHNIQUES FOR THE ANALYSIS OF TOTAL SOLUBLE PHOSPHORUS
(BASED ON RECOVERY)

Type of Sample	Unit of Expression	Method of Analysis				
		H_2SO_4 / HNO_3 Digestion[a]	Ignition 800°C	H_2O_2 Digestion		Persulfate Digestion
				Jackson [5]	Improved[d]	
Orthophosphate standard 16.3 µg PO_4-P	µg	16.3	16.3	14.6	16.3	16.3
Orthophosphate standard 16.3 µg PO_4-P in 20 gm Cl^-/ℓ	µg	----	----	----	----	16.3
Difco Tryptone 3-30µg; 0.97% P[b]	%	0.92	0.97	0.66	0.88	0.97
Difco Tryptone 3-30 µg; 0.97% P[b] in 20 gm Cl^-/ℓ	%	----	----	----	----	0.97
Difco Peptone 15-100 mg; 0.22% P[b]	%	----	0.26	0.18	0.20	----
Bay Water, Pittsburg	µg P/ℓ	58.7[a] 47.0	56.1[c]	38.5	51.9	51.9
Bay Water, Crockett	µg P/ℓ	87.2[a] 80.0	90.5[c]	66.0	81.9	79.5
Bay Water, Fort Baker	µg P/ℓ	95.0[a] 97.6	116[c]	72.5	98.5	99.3

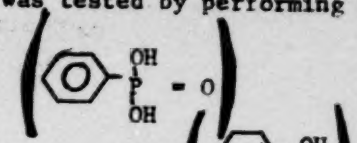
a. The values in the left column are from analyses run in unaged glassware and may be high due to contamination.

b. Values for Peptone and Tryptone supplied by Difco Company.

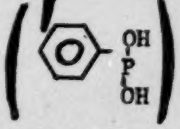
c. Several runs were made with scattered results. These values are average.

d. See text for modified method.

Menzel and Corwin [8] have tested the persulfate digestion method on several resistant organic phosphorus-containing compounds (lecithin, phosphorocholine and 5-adenylic acid) as well as on zooplankton tissue and report excellent recoveries of organic phosphorus. When these workers used this technique for the analysis of organic phosphorus in Indian Ocean water, they found that their results were somewhat low but the discrepancies were not statistically significant. During the present study the ability of persulfate to cleave P-C bonds was tested by performing the total soluble phosphorus test on

phenyl phosphonic acid 

and

phenyl phosphorous acid 

Complete recovery of phosphorus from each of these materials was obtained at the 50 µg P level. This would tend to suggest that all soluble phosphorus-containing compounds could be determined by this analytical technique.

c. Precision and Accuracy

The precision of the persulfate digestion technique for the determination of total soluble phosphorus was tested on three Bay water samples of widely differing salinity. Concentrations of total soluble phosphorus varied between about 60 µg P/ℓ to about 100 µg P/ℓ in the three samples. From Table VI it can be seen that the coefficient of variation of the technique is well within the limits of acceptability, the maximum value obtained being 1.2 per cent for the sample taken from Pittsburg. The other two samples showed a coefficient of variation of less than 1 per cent.

The use of potassium persulfate as a digestion agent contributed little to the magnitude of the reagent blank (about 0.005 absorbance units).

As noted previously, persulfate digestion gave total soluble phosphate recoveries for Bay water samples that compared well with the more drastic (and less convenient) digestion techniques.

6. Total Insoluble Phosphorus

a. Method Development

In order to release all of the phosphorus associated with the particulate material present in Bay water, it is necessary to use a drastic oxidation or digestion technique. A review of the literature for the analysis of total phosphorus in soils reveals that the most popular digestion methods involve the use

TABLE VI

REPLICATION OF PERSULFATE TECHNIQUE FOR THE ANALYSIS OF TOTAL SOLUBLE PHOSPHORUS IN SAN FRANCISCO BAY WATER

	Location of Sampling		
	Pittsburg	Crockett	Fort Baker
µg P/ℓ	49.3	79.6	100
	49.6	79.3	100
	49.6	79.6	98.5
	50.6	80.3	100
	48.6	79.0	98.5
	50.6	80.3	98.5
	49.6	79.3	100
	49.6	79.6	100
	49.3	79.0	99.2
	50.0	80.3	99.5
Mean, µg P/ℓ	49.6	79.6	99.5
Standard Deviation, µg P/ℓ	0.59	0.52	0.79
Coefficient of Variation %	1.20	0.66	0.80

of perchloric acid. The literature is replete with modifications of the perchloric acid digestion technique, all of which are undoubtedly applicable to the specific situations for which they were proposed [9]. There is a widespread acceptance that however drastic the perchloric acid digestion is made, there will still be some phosphorus-containing materials that will not release. This conclusion has been substantiated in this laboratory in previous work where it was found that by using a sodium carbonate fusion technique phosphorus yields 2-5 per cent higher than those from nitric acid/perchloric acid digestions were obtained from San Francisco Bay sediments. In recommending a perchloric acid digestion technique for the estimation of the total phosphorus content of suspended material in San Francisco Bay water, it should be realized that the results obtained by this method will be slightly lower than the actual total phosphorus content of the suspended material. This method is recommended over the sodium carbonate fusion technique because of the tedious nature of the latter and the substantial investment in platinum ware that would be required if this technique were to be applied routinely to a large number of samples.

In carrying out a perchloric acid digestion, the amount of perchloric acid used in each sample must be adjusted so that both a satisfactory digestion is ensured and also suitable acidity conditions prevail at the end of the digestion procedure. Using the amount of perchloric acid suggested in the recommended technique, it has been found that a ± 20 per cent variation in acidity can be tolerated in the digested sample mixture without significantly affecting the colorimetric estimation of released phosphorus. However, if a greater loss of acid is suffered the resulting absorbance will be erroneously low. While using the recommended conditions during the method development phase of this study, it was found that acid concentrations varied no more than ±10 to 12 per cent after digestion. Thus, of the original 21 ml perchloric acid introduced at the start of digestion, 19 ml ± 1 ml remained at the end of the digestion procedure.

In the recommended procedure, the sample is digested with concentrated nitric acid prior to the addition of perchloric acid so that easily oxidizable organic material which might react explosively with perchloric acid is removed. Since the procedure calls for the analysis of suspended material retained on a Millipore filter (which is fabricated from easily oxidizable material) it would seem essential that nitric acid be used in the digestion procedure. To further reduce the possibility of explosive oxidation of the Millipore filter

material and also to shorten the digestion procedure, the Millipore filters can be ignited with a platinum loop prior to digestion. When a dry Millipore filter is ignited in this manner it will burn rapidly leaving only a small residue. There has been some indication that the ignition of Millipore filters in this manner may cause the loss of a small amount of phosphorus from the retained solids. It is possible to avoid this loss by drying the Millipore filter and its retained suspended material in the presence of 1 ml of 5% $MgCl_2 \cdot 6 H_2O$. This procedure has the effect of fixing any released phosphorus as magnesium pyrophosphate thus preventing its volatilization. However, since the loss of phosphorus in this ignition step is quite small, and in any case the nitric/perchloric acid digestion is not an absolute measure of the total phosphorus present, the addition of $MgCl_2$ may be omitted.

b. Recommended Method

The recommended method for the analysis of total insoluble phosphorus is digestion of suspended material retained on a Millipore filter using nitric acid and perchloric acid followed by a colorimetric estimation of the orthophosphate released by digestion. By performing the analysis of total phosphorus on the suspended matter derived from the original sample by filtration, it is possible to obtain suspended material from large volumes of solutions and carry out a direct analysis of suspended phosphorus. The method also eliminates the difficulties encountered in performing perchloric acid digestions on large volumes of highly saline samples. Thus, the accumulation of salt that takes place during the reduction in volume caused by evaporation and digestion is avoided.

c. Precision and Accuracy

It is assumed that perchloric acid digestion will release and convert to orthophosphate essentially all of the phosphorus present in the small quantity of finely divided material contained in Bay water samples.

Several washed Millipore filters have been analyzed and found to contribute negligible amounts of phosphorus (See Section IV-4b). Reagent blank values of 0.002-0.005 absorbance units have typically been obtained. No loss of PO_4-P from a standard orthophosphate solution digested by the recommended method has been observed.

Replicate data for the analysis of total insoluble phosphorus on the sediment derived by Millipore filtering three samples of Bay water from widely varying points in the San Francisco Bay system are presented in Table VII. The three samples contained total insoluble phosphorus concentrations varying from about 15 µg P/ℓ up to about 110 µg P/ℓ. The

coefficient of variation was at no time greater than 3 per cent and can be kept below this acceptable value even for very low concentrations of total insoluble phosphorus, by filtering large volumes of sample (Table VI).

7. "Availability" of Insoluble Phosphorus

a. Discussion

From the figures presented in Table VII and from previous data collected in this laboratory, it can be deduced that San Francisco Bay water may contain as much, or even more, phosphorus in a suspended form than it does in the dissolved state. It is of prime importance that the availability of this suspended phosphorus to the biota of the Bay be determined, since it represents a considerable reserve of potential nutrient material.

In view of these findings an exploratory investigation was made of the "biological availability" of the phosphorus associated with the suspended matter. While the chemical techniques available for such a determination are necessarily biologically non-specific, a certain rationale can be applied to the results. The techniques are based on a solubility fractionation of the adsorbed phosphorus material. Therefore if one can chemically extract a certain amount of the total phosphorus content from a particle of suspended matter with relative ease, it may be assumed that this fraction of the phosphorus would be "more available" than the unextracted portion. Whether availability to microorganisms is reflected by this ease of chemical extraction is a debatable point and one which should be determined by growth studies on the organism and system of interest.

Several methods for the determination of the "biological availability" of phosphorus in soils have been suggested in the literature. These methods have been proposed by various workers for soils in different parts of the country. This exploratory investigation of the availability of phosphorus in the suspended solids present in San Francisco Bay water was performed using a procedure reported by Chang and Jackson [10]. These authors state that the method yields all adsorbed and loosely bound insoluble phosphorus, all aluminum phosphates and calcium phosphates and a "considerable quantity" of the iron phosphates present.

b. Method of Determination

A water sample was passed through a Millipore filter and the filter with its retained solids were then shaken in a plastic bottle with 50 ml 0.1N HCl for 30 min. using a shaker adjusted to provide moderate agitation. Ammonium fluoride (1 g) was then

TABLE VII

REPLICATION OF NITRIC ACID, PERCHLORIC ACID METHOD FOR TOTAL INSOLUBLE PHOSPHORUS IN SAN FRANCISCO BAY WATER

	Location of Sample		
	Pittsburg	Crockett	Fort Baker
Suspended Solids, mg/ℓ	74	38	10
Volume Filtered, ml	250	250	500
µg P/ℓ	108	65.6	14.4
	110	66.3	14.4
	114	64.4	13.2
	115	65.6	13.7
	114	65.6	14.7
	109	65.3	14.0
	114	66.6	14.2
	114	64.0	14.2
	115	65.3	13.8
	114	66.0	13.8
Mean, µg P/ℓ	113	65.3	14.0
Standard Deviation µg P/ℓ	2.68	0.80	0.41
Coefficient of Variation, %	2.37	1.23	2.93

added to the sample and the mixture was shaken for 1 hr. more. After the addition of 15 ml 0.8M boric acid solution (to complex any excess of F^-) and dilution to 200 ml, the samples were analyzed for orthophosphate without filtration.

c. Results of Analysis

Quadruplicate analyses of dry sediments each derived from 500 ml Bay water taken from Crockett yielded the following results: 39.4, 40.4, 40.0, and 38.8 µg "available" P/ℓ. When the same analysis was carried out on wet sediment retained on a Millipore filter, a value of 38.0 µg "available" P/ℓ was obtained. The reagent blank was negligible and an orthophosphate standard solution was completely recovered by the technique.

These data indicate that, for this sample of suspended matter, approximately 60 per cent of the total insoluble phosphorus was in the "available" form (as defined by this technique) and that the results of this analytical method were quite reproduceable. Furthermore, little difference in results was obtained whether wet or dry sediment was analyzed.

It is interesting to make a comparison between the quantity of insoluble phosphorus which might be released by merely shaking the suspended matter in neutral distilled water. Previous work in this laboratory has shown that about 0.15 mg P/g dry sediment could be extracted in this manner from silt-clay sediments from San Francisco Bay. Assuming the concentration of total insoluble phosphorus was on the order 1.5 mg P/g dry sediment this means that about 10 per cent of the insoluble phosphorus (i.e. phosphorus only slightly insoluble or loosely adsorbed on the sediment) is extractable by shaking with distilled water compared with about 60 per cent that is classified as "available" by the acid-fluoride extraction technique of Chang and Jackson [10].

V. PRESERVATION OF PHOSPHORUS FORMS

1. Literature Review

During the past two decades several workers have observed changes in the concentration of phosphorus forms in water samples during storage and have made various attempts to prevent these changes. Of the score or more variables involved in a study of the preservation of phosphorus forms; i.e., container material, size, and pretreatment; volume and type of sample material; initial filtration procedure (if any); storage temperature; concentration and form of chemical preservatives used; period of storage, and details of the phosphorus forms determined, including their concentration ranges and the methods of analysis employed; only a few are usually discussed by any given author. Also, since conditions during the experiments performed by various workers are only seldomly comparable, any explanations for the mechanisms of concentration changes observed in the phosphorus forms are not usually comparable or are often in complete disagreement.

Harvey [6], using glass storage containers, found that neither chloroform nor 0.28 N acid prevented a decrease in the total phosphorus content of sea water samples. He assumed that organic compounds, some of which contained phosphorus, were being adsorbed on the glass surfaces of the containers. His solution to this problem was to bait the samples with aluminum hydroxide on which the organic material and bacteria were adsorbed. A few ml of chloroform were also introduced into the sample. At the time of analysis the aluminum hydroxide was dissolved with sulphuric acid in order to resuspend the adsorbed materials. Thorium carbonate was also found to be a satisfactory material for the adsorption of organic material and bacterial cells.

In later work Harvey [11] expanded his recommendations for satisfactory preservation of phosphorus forms in sea water. Notably, he recommended that the samples should be saturated with chloroform and heated to 56°C to inactivate the enzymes in the sample to which a substance known as nuclianate* had previously been added. Using these methods of preservation Harvey [11] found that, at 20°C, there was less than 10 per cent increase in PO_4-P concentrations during a 24-day storage period. Contrary to the results presented in a later experimental section of the present report, Harvey felt that saturation of the sample with chloroform and refrigeration was a satisfactory long-term preservation method. Harvey also recommended that soft glass containers should not be used to store samples which were to be analyzed for the various forms of phosphorus.

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* A nucleic acid-albumin compound derived from yeast cell nuclei--Merck Index pp. 739 and 957.

Murphy and Riley [12] filtered sea water samples immediately after collection and stored them in dark soda-glass bottles at about 20°C for one month. When no preservative was added to the samples, a decrease of about 10 per cent was found in the concentration of PO_4-P. The decrease was attributed to enzymatic and bacterial action on organic phosphates. The use of 1 ml chloroform/150 ml sample was found to be a satisfactory preservation method for concentrations of PO_4-P in the region of 30 µg/ℓ. The addition of flouride ion was found to be an adequate method of preservation but suffered from the disadvantage that flouride interfered with the colorimetric analysis of the phosphate. Efforts by these workers to use aluminum hydroxide or thorium carbonate bait as employed by Harvey [6] produced "high and variable" results. It was also found by Murphy and Riley [12] that polyethylene bottles strongly adsorbed phosphate from the water samples.

Strickland and Parsons [3] recommended quick freezing to -20°C within 30 minutes of sample collection, using a 40 per cent glycol bath. These authors recommended that preservation should be carried out by this method if the sea water samples were to be analyzed at periods exceeding 1 hour after sample collection. According to these authors, this freezing technique stabilized the phosphorus content of the samples for many months. Strickland and Parsons [3] recommended the use of polyethylene containers for the storage of samples to be later analyzed for phosphorus forms because they found that, over a period of time, pyrex glass containers released phosphorus into the samples, even after rigorous pretreatment by washing with dilute acid. Samples for total phosphorus analysis, according to Strickland and Parsons [4], may be stored in pyrex glassware for short periods at room temperature. They recommended an addition of one or two drops of concentrated hydrochloric acid to each 50 ml of sample and the use of parafilm sheeting in the place of corks or rubber stoppers.

Collier and Marvin [13] also favored the use of quick freezing for the preservation of samples to be analyzed for phosphorus forms. By quick freezing immediately after sample collection and then storing at sub-zero temperatures in polyethylene bottles, they found that no change in the concentration of orthophosphate took place during 8 days.

In a recent publication, Heron [14] discussed the use of polyethylene bottles, with and without chemical preservatives, for the storage of centrifuged lake water on which PO_4-P determinations were to be made. Working in the 2-5 µg PO_4-P/ℓ range, 7 ml of chloroform and unspecified quantities of toluene and 1,2-dichloroethane were each found to be unsatisfactory preservatives. In this work, the temperature of storage was not specified. The least promising of the three preservation methods tried by Heron [14] was chloroform. Samples preserved with chloroform showed a sharp increase in orthophosphate concentration over the first week of storage which was followed by a sharp decrease during the next 5 weeks. Quick freezing of samples filtered through sintered glass filters and stored in polyethylene bottles at deep freeze

temperatures, showed no change in orthophosphate after 8 days. Since there was no immediate increase in orthophosphate concentration, Heron [14] states that there is an indication that cell rupture by the quick freezing process did not take place. During the course of his research, Heron [14] became convinced that the phosphorus adsorbed on the surface of polyethylene containers was insignificant but that the changes in phosphorus concentration observed during storage in polyethylene bottles was due to the activity of a layer of bacteria present on the walls of the containers. This conclusion is in disagreement with the work of Murphy and Riley [12] who placed a sheet of polyethylene in an orthophosphate solution, rinsed it free in distilled water, and showed that the decrease in orthophosphate concentration of the solution could be completely accounted for by the orthophosphate which had been adsorbed on the polyethylene sheet.

Recognizing that quick freezing was an inconvenient process, Heron recommended that polyethylene sample bottles be pretreated with an I_2KI mixture to eliminate subsequent bacterial action on their surfaces. Unfortunately, the use of this substance produced interferences in the chemical determination of orthophosphate.

In summary, it can be said that polyethylene containers seem to be unsatisfactory for the prolonged storage of samples which will later be analyzed for phosphorus forms. It also seems that chloroform is not a satisfactory preservative for phosphorus forms and that some chemical additive in conjunction with freezing at sub-zero temperatures would seem to be the most likely method of preservation.

2. Conduct of Preservation Study

a. Sampling Methods

Five 5-gallon containers were filled with Bay water taken from the vicinity of Crockett and were brought immediately to the laboratory. The water samples contained 42 mg suspended solids/ℓ and 7.7 gm Cl^-/ℓ. Within 1 hour of arrival at the laboratory, the 5-gallon samples were treated with the specified chemical preservative where applicable, split into 1-ℓ portions and placed in previously acid-washed and thoroughly rinsed pyrex reagent bottles. The samples which were to be frozen were only partially filled to prevent breakage caused by expansion during the freezing process.

b. Preservation Methods

The preservation methods investigated during this study are summarized in Table VIII.

TABLE VIII

METHODS OF PRESERVATION EMPLOYED

	Temperature	Preservative
1	4°C	--
2	4°C	40 mg Hg^{++}/ℓ
3	4°C	5 ml $CHCl_3/\ell$
4	-10°C	--
5	-10°C	40 mg Hg^{++}/ℓ

c. Analytical Methods

On the day that the samples arrived in the laboratory, a complete set of phosphorus analyses was run on the water from each of the large containers to obtain an initial value for the concentration of the various phosphorus forms. The analyses performed were for soluble orthophosphate, total soluble phosphorus, and total suspended phosphorus.

During the 31-day course of this study, five further sets of analyses were carried out on each set of samples. Prior to analysis, the samples were brought to room temperature quickly by immersing them in a hot water bath. Immediately thereafter they were filtered through HA Millipore filters. Analyses were performed in duplicate for each form of phosphorus. The analytical techniques used were those recommended in the first section of this report and outlined in Appendix I.

3. Results of Preservation Study

The results of the preservation study are presented in Tables IX through XIII and Figures 1 through 5.

a. Storage at 4°C Without Added Preservative

Storage at 4°C without the addition of any preservative failed to hold the concentration of soluble orthophosphate constant. There was a gradual increase in soluble orthophosphate and total soluble phosphorus (which could be accounted for by the increase in soluble orthophosphate) over the 31-day period of experiment. There was a corresponding decrease in the total insoluble phosphorus of the same magnitude as the increase in total soluble phosphorus.

b. Storage at 4°C with the Addition of Chloroform

Samples stored at 4°C with chloroform added showed a large decrease in soluble orthophosphate and in total soluble phosphorus after about 5 days storage. Thus, after about 13 days of storage the soluble orthophosphate had decreased by between 30-40 per cent. During the first 8 days of the preservation study there may have been a small increase in soluble orthophosphate but the differences were too small to make any firm conclusions. The total insoluble phosphorus showed a rapid increase between days 5 to 13 of the preservation study, followed by a slower increase throughout the remainder of the period.

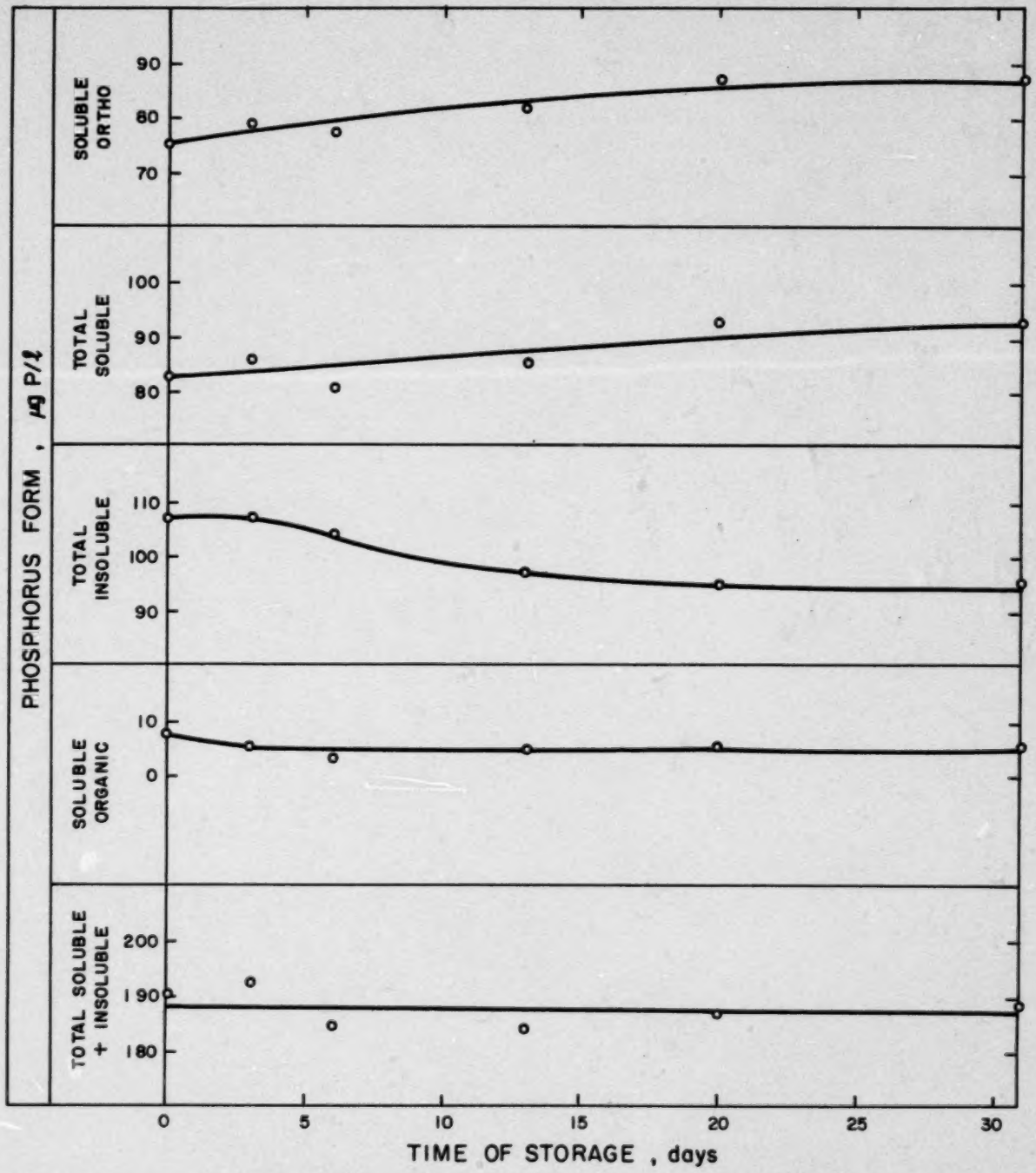


FIGURE 1. CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT 4°C WITHOUT THE ADDITION OF PRESERVATIVE

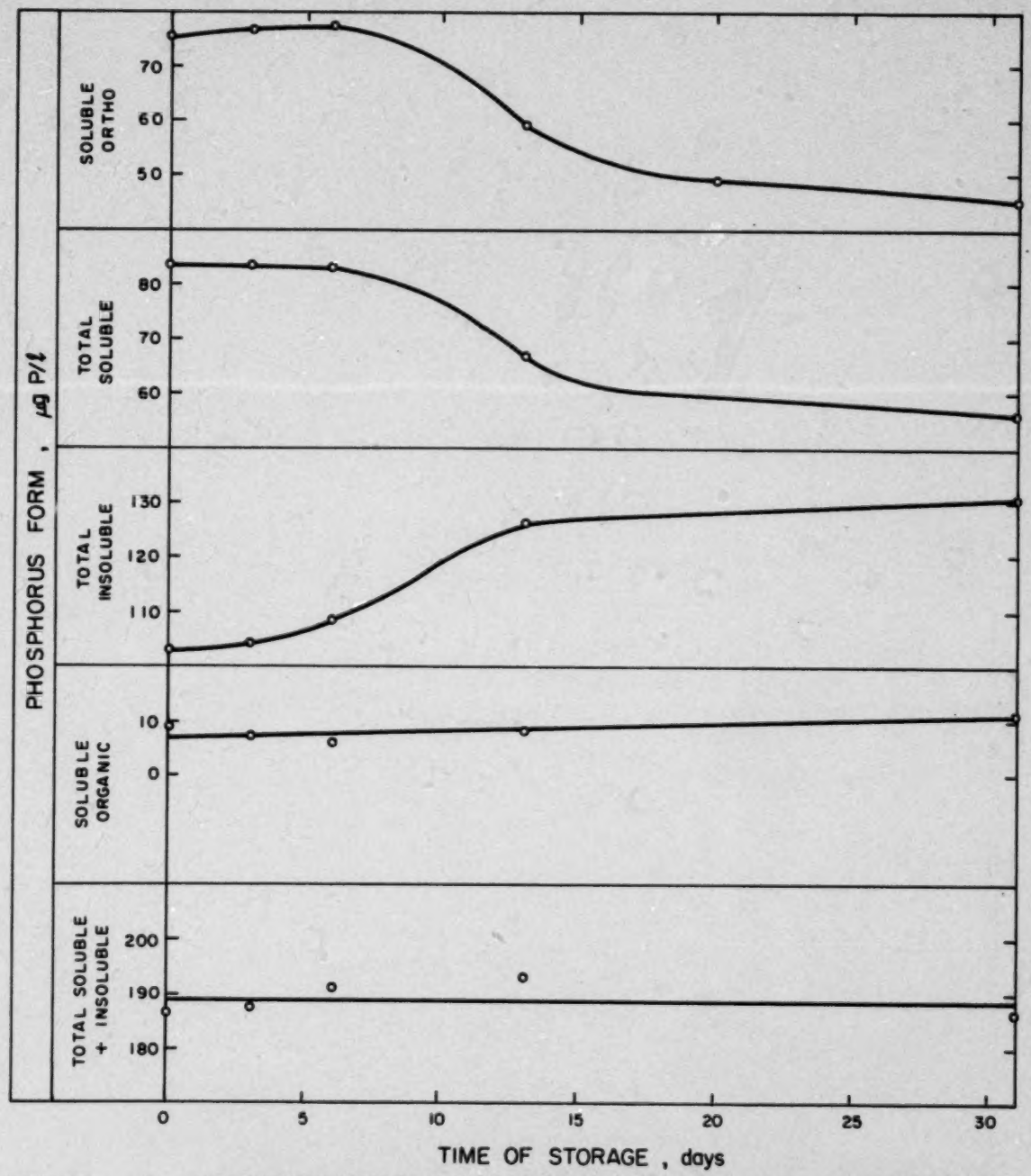


FIGURE 2. CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT 4°C IN THE PRESENCE OF CHLOROFORM

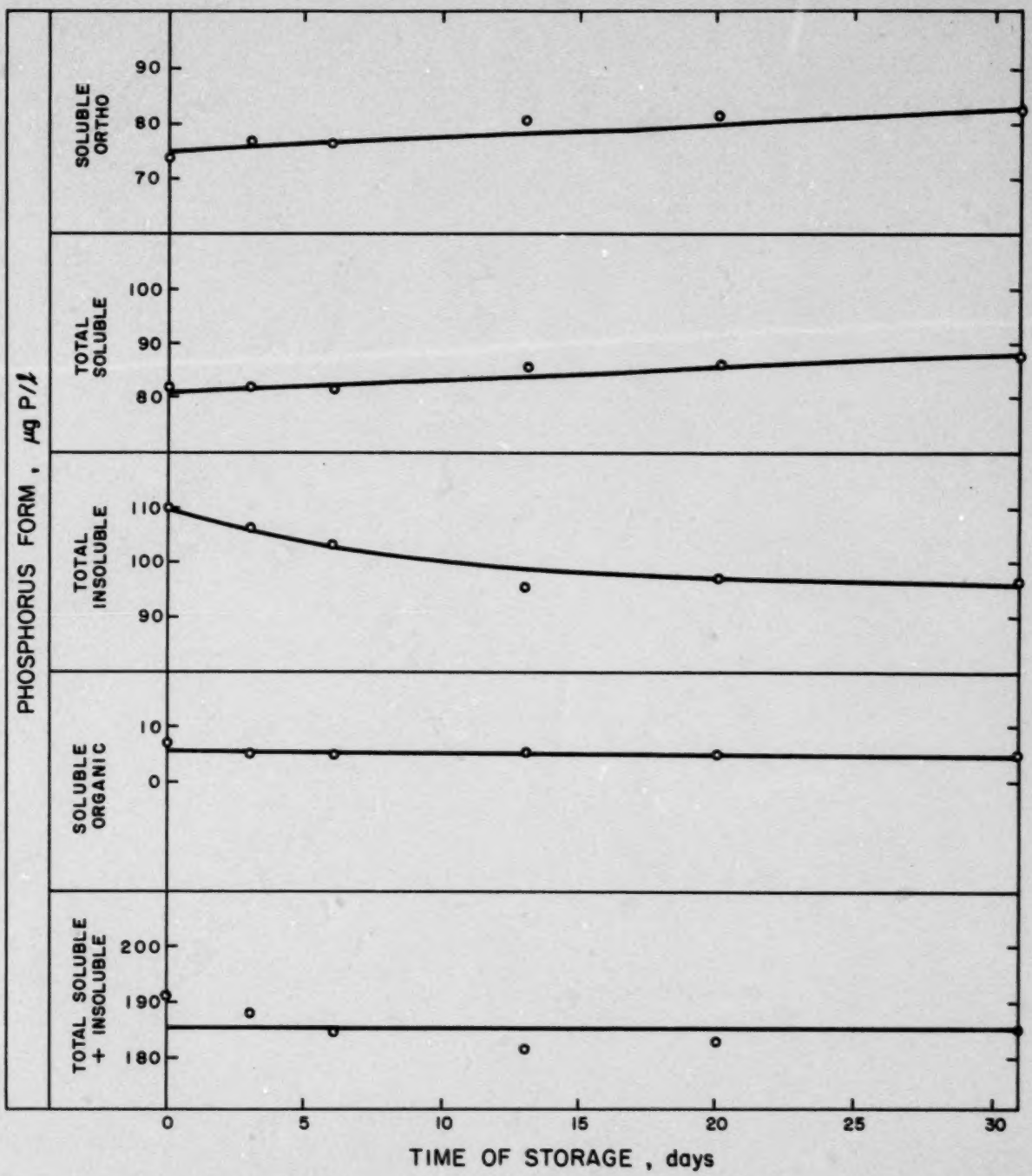


FIGURE 3. CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT 4°C IN THE PRESENCE OF MERCURY PRESERVATIVE

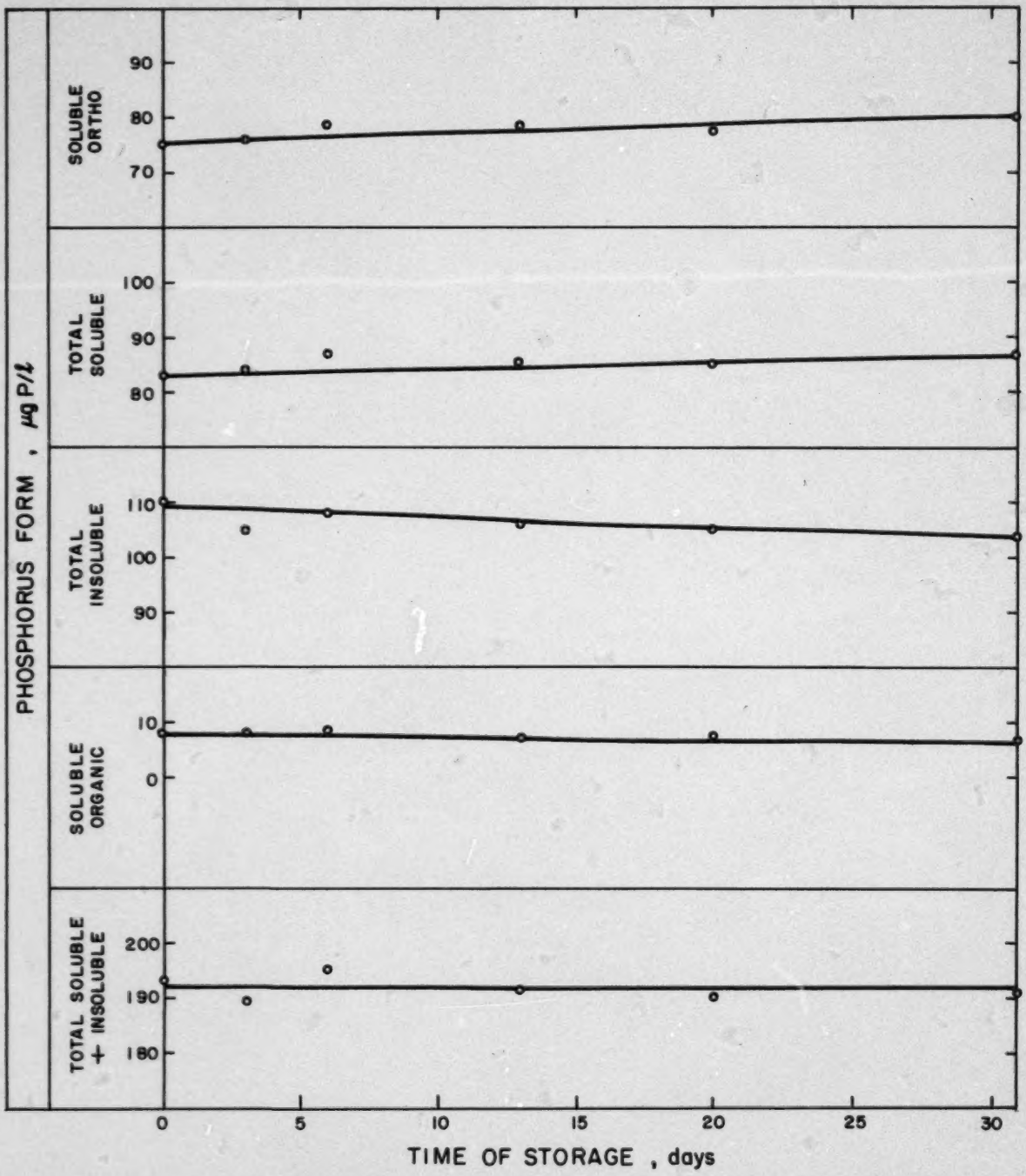


FIGURE 4. CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT -10°C WITHOUT THE ADDITION OF PRESERVATIVE

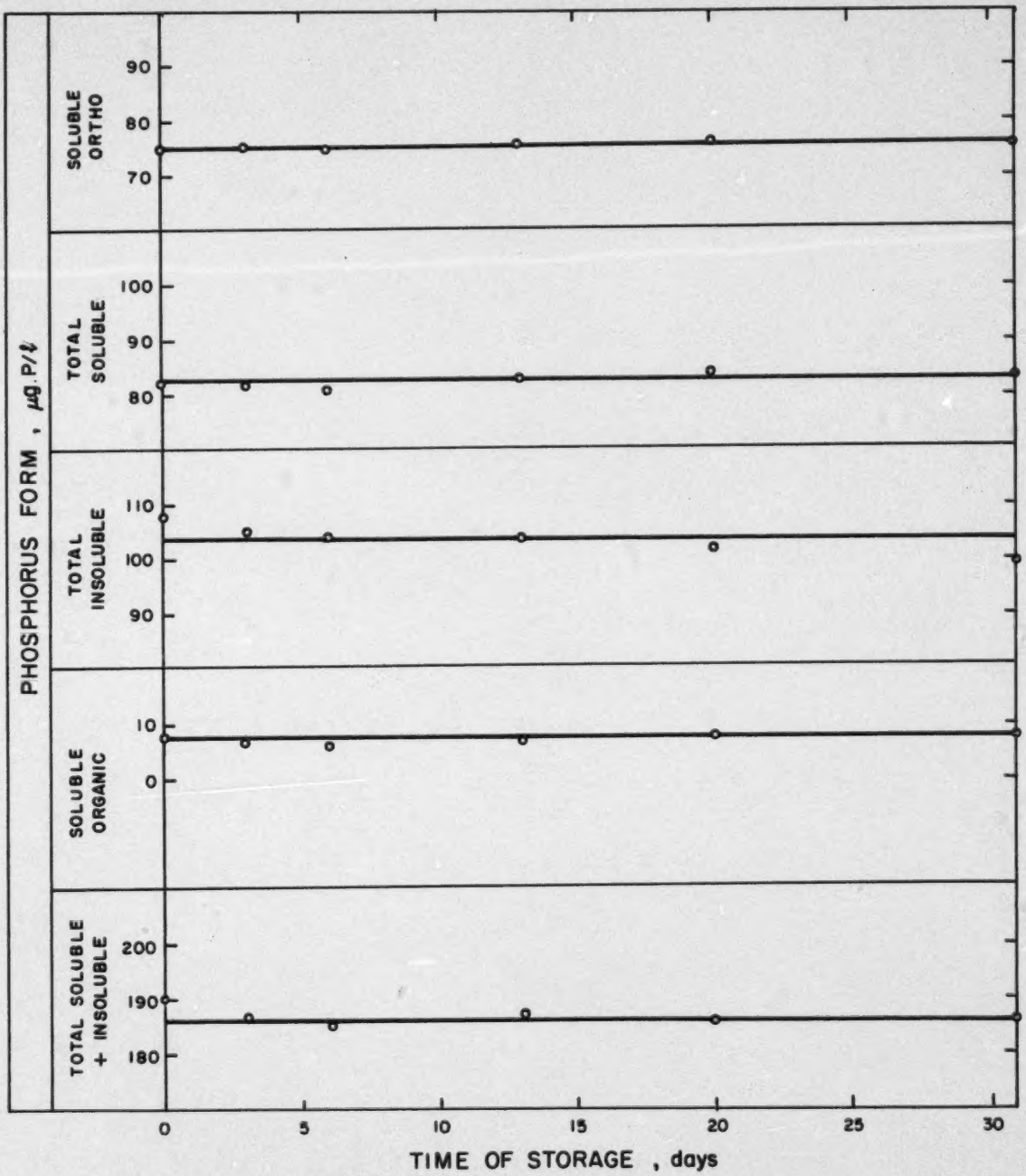


FIGURE 5. CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT -10°C IN THE PRESENCE OF MERCURY PRESERVATIVE

TABLE IX
CONCENTRATION OF PHOSPHORUS FORMS
IN SAN FRANCISCO BAY WATER DURING
STORAGE AT 4°C WITHOUT THE
ADDITION OF PRESERVATIVE

Phosphorus Form	Time of Storage, Days					
	0	3	6	13	20	31
	µg P/ℓ					
Soluble Ortho	75.2	78.8	77.2	82.0	87.0	87.0
Soluble Organic	7.6	6.6	3.4	4.7	6.0	5.9
Total Soluble	82.8	85.4	80.6	86.7	93.0	92.9
Total Insoluble	107	107	104	97.4	94.8	95.8
Total Soluble and Insoluble	190	192	185	184	188	189

TABLE X

CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT 4°C IN THE PRESENCE OF CHLOROFORM

Phosphorus Form	Time of Storage, Days					
	0	3	6	3	20	31
	µg P/ℓ					
Soluble Ortho	75.0	76.2	77.2	59.0	49.0	44.8
Soluble Organic	8.2	6.7	5.4	7.7	19.0*	10.8
Total Soluble	83.2	82.9	82.6	66.7	68.0*	55.6
Total Insoluble	103	104	108	126	140.5*	130.4
Total Soluble and Insoluble	186	187	191	193	209*	186

*Contaminated sample; these values are not shown on Figure 2

TABLE XI

CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT 4°C IN THE PRESENCE OF MERCURY PRESERVATIVE

Phosphorus Form	Time of Storage, Days					
	0	3	6	13	20	31
	µg P/l					
Soluble Ortho	74.3	77.0	76.0	80.5	81.2	82.8
Soluble Organic	7.1	4.8	5.0	5.5	5.1	5.1
Total Soluble	81.4	81.8	81.0	86.0	86.3	87.9
Total Insoluble	110	106	103	95.4	97.1	97.2
Total Soluble and Insoluble	191	188	184	181	183	185

TABLE XII

CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT -10°C WITHOUT THE ADDITION OF PRESERVATIVE

Phosphorus Form	Time of Storage, Days					
	0	3	6	13	20	31
	µg P/ℓ					
Soluble Ortho	75.0	76.2	78.5	78.5	77.5	80.0
Soluble Organic	8.0	8.2	8.5	7.1	7.8	7.0
Total Soluble	83.0	84.4	87.0	85.6	85.3	87.0
Total Insoluble	110	105	108	106	105	104
Total Soluble and Insoluble	193	189	195	192	190	191

TABLE XIII

CONCENTRATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATER DURING STORAGE AT -10°C IN THE PRESENCE OF MERCURY PRESERVATIVE

Phosphorus Form	Time of Storage, Days					
	0	3	6	13	20	31
	µg P/l					
Soluble Ortho	74.5	75.2	75.0	75.8	76.0	75.6
Soluble Organic	7.9	6.8	6.3	7.6	7.9	7.0
Total Soluble	82.4	82.0	81.3	83.4	83.9	82.6
Total Insoluble	108	105	104	104	102	99.5
Total Soluble and Insoluble	190	187	185	187	186	182

c. Storage at 4°C With Mercury Preservative

Using this method of preservation a pattern of change in the phosphorus forms, similar in character but slightly less pronounced than that obtained without any added preservative, was obtained. Thus soluble orthophosphate increased steadily from about 74 µg/l to about 83 µg/l after 31 days of storage while the total insoluble phosphorus concentration dropped from 110 µg/l to about 97 µg/l throughout the storage period.

d. Storage at -10°C Without Added Preservative

A similar trend to that observed in the samples preserved with mercury and those stored at 4°C without added preservative was observed using this method of preservation. However, the magnitude of the change of phosphorus forms was much less than in these two methods. Thus, soluble orthophosphate increased from 75 µg/l to 80 µg/l while the total insoluble phosphorus decreased from 110 µg/l to 104 µg/l over a 31-day storage period.

e. Storage at -10°C With Mercury Preservative

Storage at -10°C with the addition of 40 mg Hg^{++}/l as a preservative held all forms of phosphorus for a period of 31 days without any significant change in concentration.

In none of the methods of preservation studied was there a statistically significant change in soluble organic phosphorus.

4. Discussion of Results

From the results presented above, there is an unambiguous choice of preservation method for San Francisco Bay water samples which must be stored for prolonged periods prior to analysis. The method of choice is deep freezing (at -10°C) in the presence of 40 mg Hg^{++}/l. If the samples are to be analyzed for constituents other than the various forms of phosphorus, then the recommended concentration of mercury might possibly interfere with analytical techniques. If this is the case, then the technique of preservation to be recommended would be deep freezing (to -10°C) in the absence of Hg^{++}. Using the latter techniques, it should be possible to hold the concentrations of the various forms of phosphorus to within 2-3 percent of their initial values for a period of about 1 week. For samples which must be preserved for longer periods this method of preservation cannot be highly recommended. If facilities for freezing samples are not available and the use of

chloroform is not permitted (for example, this material will interfere with ammonia determinations made by nesslerization), then storage at 4°C might possibly be used for periods of a week or less.

In summary, it may be said that each of the preservation techniques has some merit for storage over short periods of time (less than one week) but that for longer periods of storage the only satisfactory method is preservation by freezing to -10°C in the presence of 40 mg Hg^{++}/ℓ.

VI. CONCLUSIONS AND RECOMMENDATIONS

1. Analytical Techniques

Techniques for the analysis of soluble orthophosphate, total soluble phosphorus, and total insoluble phosphorus have been developed that are suitable for the analysis of estuarine waters. The methods have been tested, with success, on samples from three areas of San Francisco Bay which are typical of the ocean, estuarine, and fresh water environment.

The recovery and precision of the various analytical techniques at levels commonly encountered in San Francisco Bay waters have been determined and are presented in this report. All techniques gave excellent replication with no coefficient of variation for any technique being in excess of 3 per cent.

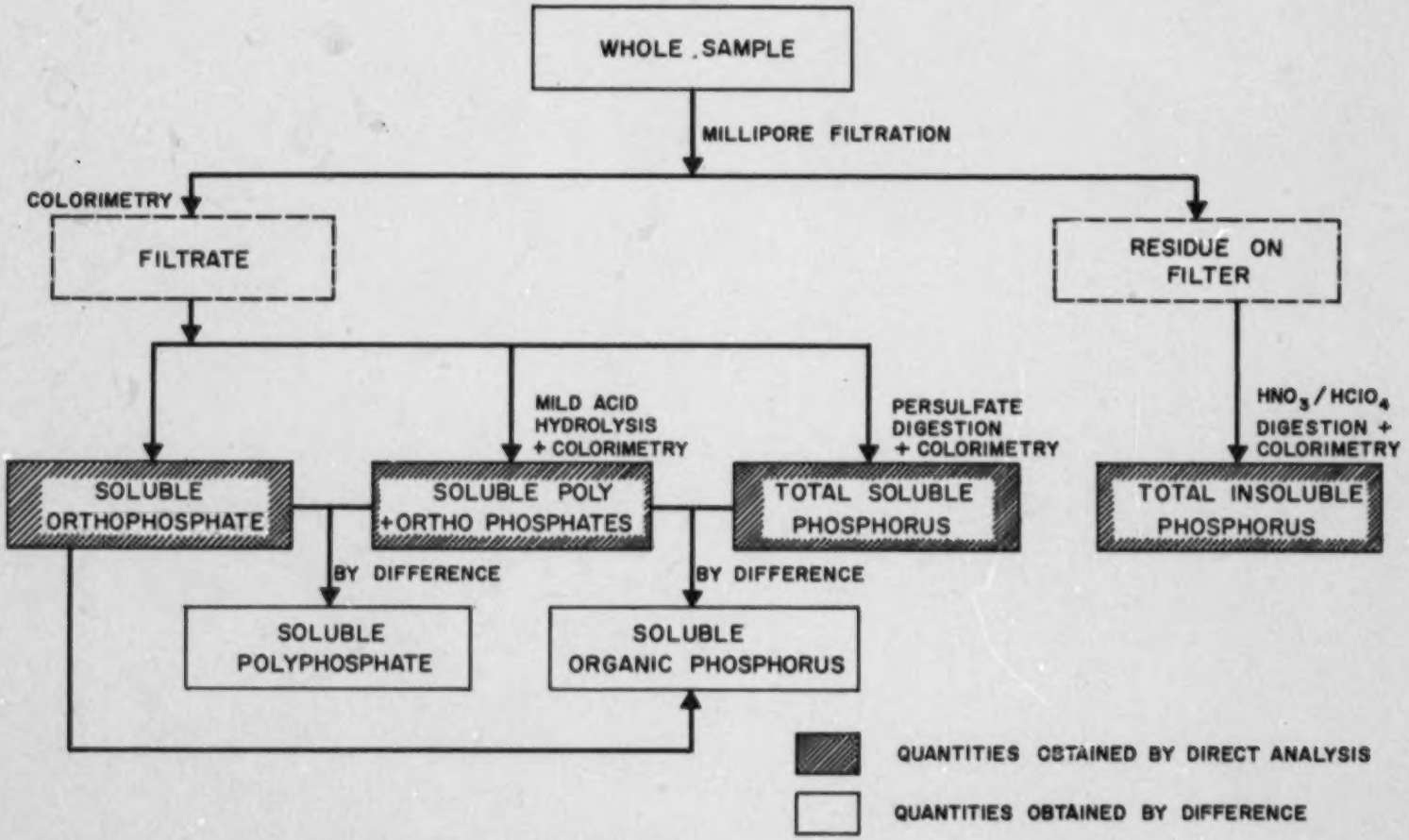
2. Scheme of Analyses

Based on the methodology presented in this report, an analytical scheme for the differentiation of the various phosphorus forms in estuarine waters was developed and is presented in Figure 6.

3. Availability of Insoluble Phosphorus

Very limited work on the availability of the insoluble phosphorus contained in suspended solids was carried out. About 60 per cent of the phosphorus present was classified as "available" by the technique of Chang and Jackson [10] while typically for Bay sediments only about 10 per cent is released by prolonged shaking in distilled water.

It is strongly recommended that further work be carried out to determine the mode and extent of phosphorus interchange between suspended material or sediment and water in the environment of the Bay. Coupled with such work, a study of the biological availability of various phosphorus forms, measured in terms of their ability to stimulate growth, should be made. The aim of



COLORIMETRY = BENZENE / ISO-AMYL ALCOHOL EXTRACTION FOLLOWED BY STANNOUS CHLORIDE REDUCTION OF EXTRACTED PHOSPHOMOLYBDIC ACID TO MOLYBDENUM BLUE

FIGURE 6. ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS IN SAN FRANCISCO BAY WATERS

such work should be to tailor a chemical technique that would selectively determine a particular phosphorus form in rough approximation to its producing potential.

4. Preservation Techniques

The procedure recommended for preservation of phosphorus forms in San Francisco Bay water will depend on whether other analyses are to be carried out on the samples and also on the time of storage prior to analysis.

For long periods of storage the only satisfactory technique is storage at -10°C in the presence of 40 mg Hg^{++}/ℓ. For shorter storage periods, e.g. less than one week, it could conceivably be possible to use a 4°C storage temperature either in the presence of 5 ml chloroform/ℓ or 40 mg Hg^{++}/ℓ or in the absence of any preservative. These storage procedures cannot be recommended for extremely precise or critical work.

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APPENDIX I

RECOMMENDED ANALYTICAL

PROCEDURES

A. WASHING OF GLASSWARE

Synthetic detergents containing polyphosphates, and glassware cleaners containing these compounds should not be used on any glassware or other apparatus used in the analyses of any of the forms of phosphorus. Standard Methods [ ] recommends that washing in hot dilute HCl followed by thorough distilled water rinsing should be used for producing a clean glass surface free of adsorbed phosphates. This procedure was used throughout this investigation and has given satisfactory results.

B. SOLUBLE ORTHOPHOSPHATE

1. Apparatus

1.1 Spectrophotometer; Bausch and Lomb Spectronic 20 or equivalent with adaptor for 1 inch cells.

1.2 Separatory Funnels; 500 ml, pear shaped, with teflon plug.

1.3 Volumetric Pipets; 25 ml with slightly enlarged tip for rapid transfer.

1.4 Volumetric Flasks; 50 ml.

1.5 Suction-Type Pipettors.

1.6 Millipore Filter Apparatus.

1.7 Millipore Filters; Type HA, 0.45 μ diameter pores. Prepare filters by soaking in distilled water (40 filters/liter) for 24 hr. prior to analysis. If the distilled water is changed after 1 hr. of soaking, a further three hours soaking in fresh distilled water is adequate.

2. Reagents

2.1 Stock Standard Phosphate Solution; Hellige reagent, or dry reagent grade KH_2PO_4 at 110°C for 1 hr. and then dissolve 0.467 g in distilled water and make up to 1 liter.

(1 ml $\equiv$ 1 mg P)

2.2 Working Standard Phosphate Solution; Dilute stock standard phosphate solution (2 ml) to 1 liter with distilled water.

1 ml $\equiv$ 2 μg P.

2.3 Sulfuric Acid Solution; 8 N.

2.4 Sulfuric Acid Solution; 1 N.

2.5 Neutral Ammonium Molybdate Solution; Dissolve 100 g reagent grade $(NH_4)_6\ MO_7\ O_{24}\cdot\ 4H_2O$ in distilled water and dilute to 1 liter. Store in a tightly stoppered, amber bottle.

2.6 Acidic Molybdate Reagent; Mix equal parts of Reagent 2.3 and Reagent 2.5. Keep in a tightly stoppered, amber bottle.

2.7 Benzene-Isoamyl Alcohol Solvent; Mix equal volumes of reagent grade benzene and isoamyl alcohol.

2.8 Stock Stannous Chloride Solution; Dissolve 10g reagent grade $SnCl_2.2H_2O$ in 25 ml conc. HCl. Keep in a tightly stoppered, amber bottle and prepare fresh each two weeks.

2.9 Dilute Stannous Chloride Solution; Add 0.5 ml of stock stannous chloride solution to 100 ml of 1N H_2SO_4. Prepare freshly each day.

2.10 Methyl Alcohol; Reagent grade.

2.11 Alcoholic-Sulfuric Acid Solution; Add 20 ml of conc. H_2SO_4 to 980 ml methanol.

3. Procedure

Place 200 ml of filtered bay water (or an aliquot made up to 200 ml with distilled water) in a 500 ml separatory funnel. Add 50 ml of the benzene-isoamyl alcohol solution and 50 ml of the acid-molybdate reagent. Shake vigorously for 1 minute and allow the layers to separate. Using a 25 ml volumetric pipette with a suction-type pipettor, draw off 25 ml of the organic layer and place it in a 50 ml volumetric flask. Add about 15 ml alcoholic-sulfuric acid solution and swirl. Add 1 ml of the stannous chloride reducing agent and make up to the mark with more alcoholic-sulfuric acid solution. Mix well and read the absorbance within 1-3 minutes at 630 mμ in a 1 inch colorimeter cell after zeroing the instrument on distilled water.

If a series of samples are to be analyzed then the above procedure may be interrupted after the extraction step and before the stannous chloride reduction step for periods of up to 30 minutes so that several extracts may be collected for subsequent reduction and colorimetric estimation.

A standard and blank reagent should be run daily.

4. Calculation

Beers law applies between at least 0-50 μg P

Thus

$$\mu g\ PO_4\text{-}P/l = \frac{\mu g\ PO_4\text{-}P \text{ in standard} \times \text{Absorbance of sample}_X \times 1000}{\text{Absorbance of standard} \times \text{Sample volume (ml)}}$$

5. Precision

The coefficient of variation for the soluble orthophosphate technique was between 1-1.4 per cent for the analysis of bay waters containing between about 50-100 µg P/l.

C. TOTAL SOLUBLE PHOSPHORUS

1. Apparatus

1.1 Erlenmeyer Flasks, 300 ml, with loose-fitting plastic caps to cover the flasks while autoclaving. Age the flasks before use by autoclaving with potassium persulfate solution as in the technique of analysis.

1.2 Autoclave - Capable of developing 20 psi.

1.3 Spectrophotometer; Bausch and Lomb Spectronic 20 or equivalent with adaptor for 1 inch cells.

1.4 Millipore Filtration Apparatus.

1.5 Millipore Filters; Type HA, 0.45 μ diameter pores. Washed as in Appendix I, B, 1.7.

2. Reagents

2.1 Potassium Persulfate Solution; Dissolve 5 g reagent grade $K_2S_2O_8$ in 100 ml distilled water. Prepare fresh daily.

2.2 Reagents and Apparatus for Colorimetric Estimation of Orthophosphate. See Appendix I, B.

3. Procedure

Place a 150 ml volume of filtered sample and an aged 300 ml Erlenmeyer flask and add 25 ml of the 5% $K_2S_2O_8$ solution. Cover the flasks with the loose-fitting plastic caps and autoclave at 15-20 psi (125-150°C) for 1 hour. Cool and dilute to 200 ml with distilled water then perform a colorimetric analysis for ortho-phosphate as described in Appendix I, B, 3.

4. Calculation

Same as for soluble orthophosphate P but express the results as μg Total Soluble P/l. Soluble organic P is determined as:

Total Soluble P - Soluble orthophosphate P = Soluble Organic P

5. Precision

A coefficient of variation of between 0.7-1.2 per cent was obtained when samples containing between 50-100 μg P/l were analyzed.

D. TOTAL INSOLUBLE PHOSPHORUS

1. Apparatus

1.1 Hot Plate; Lindberg or equivalent; 30 x 50 cm heating surface is adequate.

1.2 Erlenmeyer Flasks; 250 ml, wide mouth and aged by acid treatment such as encountered in the analytical procedure.

1.3 Platinum Loop.

1.4 Safety Shield.

1.5 Safety Goggles.

1.6 Spectrophotometer; Bausch and Lomb Spectronic 20 or equivalent with adaptor for 1 inch cells.

1.7 Millipore Filter Apparatus.

1.8 Millipore Filters; Type HA, 0.45 μ diameter pores. Washed as in Appendix I, B, 1.7.

2. Reagents

2.1 Nitric Acid, concentrated, reagent grade.

2.2 Perchloric Acid; $HClO_4 \cdot 2H_2O$; purchased as 70-72% $HClO_4$, reagent grade.

2.3 Reagents for colorimetric estimation of orthophosphate; See Appendix I, B.

3. Procedure

The selection of the proper sample volume to be filtered depends on the suspended solids content of the sample and the phosphorus content of the suspended matter. The samples used in the present study contained about 1.5 mg P/g of dry sediment. If this value can be regarded as representative, little difficulty should be encountered estimating the proper sample volume for analysis. A "rule of thumb" used in this study was to filter 250 ml of a sample which appeared turbid, 50-100 ml from heavy suspensions, and as much as a liter for relatively clear water.

Place the filters with retained sediment face down in a 250 ml wide-mouth erlenmeyer flask and allow to dry in air or at 105°C either in the absence or presence of 1 ml of 5% $MgCl_2$ $6H_2O$ solution. After drying, touch the edge of the filter with a hot platinum loop. Usually most of the Millipore filter material will combust readily leaving a light ash and sediment residue. Take all possible precautions to prevent the loss of the light ash material from the erlenmeyer flask. Add 10 m conc. HNO_3 and heat on a hot plate in a fume hood until the volume is reduced to 2-3 ml. (Since membrane filters are fabricated from cellulose esters which are soluble in concentrated HNO_3, it is possible to omit the ignition step and add an extra 10 ml conc. HNO_3 and perform a longer digestion to destroy the membrane filter material.)

Remove the flask from the hot plate and cool. Set up a safety shield and add 21 ml $HClO_4$ solution. Heat until dense fumes of $HClO_4$ are evolved. Digest 10-15 minutes longer and then remove the flasks from the hot plate. The volume of acid remaining should be between 19 ml $\pm$ 1 ml. However, if the volume of acid is reduced below these limits, $HClO_4$ may be added prior to colorimetric analysis. IN NO EVENT SHOULD SAMPLES BE ALLOWED TO DEVELOP ANY DRY SPOTS, MUCH LESS EVAPORATE TO DRYNESS, BECAUSE THIS IS A COMMON CAUSE OF EXPLOSION. SAFETY GLASSES OR GOGGLES MUST BE WORN AT ALL TIMES WHEN WORKING WITH PERCHLORIC ACID. Since the average hot plate does not heat uniformly over its whole surface, correct timing for digestion may be problematical when large numbers of samples are digested on one hot plate. Similarly the rates of heating of individual hot plates vary considerably so that each laboratory should establish its own digestion times to produce the desired final volume of acid.

Cool the flasks to room temperature and carefully add distilled water to a final volume of 225 ml. Perform a colorimetric analysis of orthophosphate as described in Appendix I, B except that since the sample already contains the proper acid concentration use 25 ml of 10% neutral ammonium molybdate in place of the 50 ml of acid molybdate used in the orthophosphate method.

4. Calculation

Use the same calculation as for soluble orthophosphate but express the results as µg Total Insoluble P/l.

5. Precision

A coefficient of variation of less than 3 per cent was obtained for samples containing between 7 and 28 µg P.